this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB3]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT4]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA has, for decades, relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT5]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

1 Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.*, a dose or concentration that marks the beginning of a low-dose

Commented [HT6]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Exposure Assessment

Commented [HT7]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments…include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value is 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

Commented [HT8]: (U.S. EPA, 1994).

Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most

often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the “unreasonable risk” determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

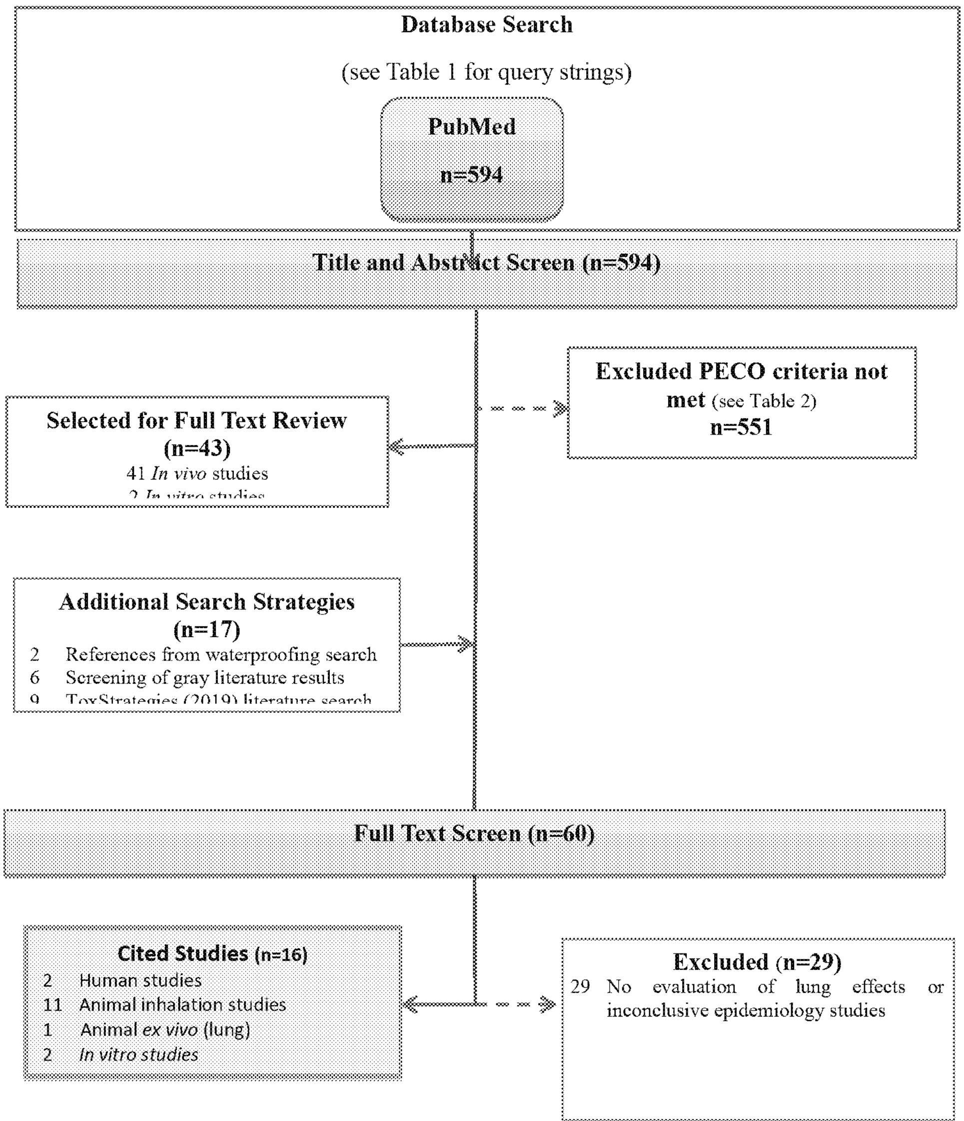
The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

Commented [ST9]: This section needs updating following final disposition of gray lit and Updated Literature Search.

Scheme 1. Literature search and screening flow diagram for surfactants

Commented [ST10]: The tally of Cited and Excluded references from the bottom of the figure includes the PubMed results only. These boxes need to be updated following disposition of 6 studies from the gray lit. search and 9 studies from the Updated Literature Search.



Category Boundaries

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

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[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and the assessment should be conducted on the predominant ~~or both~~ components. The non-ionized fraction for acids/bases should be calculated as follows:

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{pH-pKa})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{pKa-pH})$$

Where the pH represents the physiological pH in the lung (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine). ~~A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.~~

Commented [KA11]: Should this sentence be deleted?

Commented [OS12]: Todd will update to simplify, show equation,
Aromatic amines is an aniline, ph7

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

[INSERT TABLE X]

Commented [ST13]: Temp?

Commented [OS14R13]: Mike/Wayne to include clarifying text

Commented [HT15]: Need temp here to compare

Commented [HT16]: REMOVE CITE TO EGHE, ADD FOR TWEEN 80

Commented [ST17]: Not in Mike's Table

Commented [ST18]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"] this paper shows ST to be a lot higher

Commented [ST19]: Concentration?

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Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Commented [ST22]: Add the following, based on Updated Literature Search?
Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS23]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

<u>Nonionic Surfactants</u>

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed

Commented [OS24]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

In rodent models, irritation and inflammatory effects on the respiratory tract has been observed with varying degrees of severity. Acute inhalation exposure to Polysorbate 20 via nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/l (5,100 mg/m^3) did not observed in mortalities, clinical signs, or abnormalities in the gross pathology[3]. Using MPPD modeling, the total lung deposition mass was calculated to be 6.6E+4 µg. A respiratory irritation study was conducted on a mixture containing Nonidet in male Webster mice using the ASTM Method E981 where animals were exposed for 3 hours to concentrations of 12, 22, 51, 118, and

[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3"]

134 mg/m^3 (Alarie and Stock, 1992, unpublished). Signs of respiratory irritation was observed in animals at the three highest concentrations as indicated by increased respiratory frequency without an increase in pulmonary edema or lung weight. An acute inhalation exposure study in Syrian hamsters to 3.0 mg/l of Triton X-100 to varying exposure durations reported that lung deposition of Triton X-100 corresponded to mortality with an LD50 of 1300-2100 µg (Damon et al., 1982). The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways and lungs in these animals were relatively free of serious pathologies. The authors hypothesized that that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the deposited chemical resulted in a large concentration of the chemical on the laryngeal mucosa. Finally, in the only repeated dose inhalation exposure identified for nonionic surfactants, a 2-week repeated dose inhalation study was conducted on Triton X-100 in male and female Sprague-Dawley rats to 5.3 mg/m^3 (MMAD 1.8 µm, GSD 1.8µm) for 6 hours/day, 5 days/week (Bio/dynamics, Inc. 1992[4].) Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge.

Commented [SK25]: It is unclear to me if the other tested concentration should be included since it is a 70% mixture.

In vitro studies of surfactant effects on cell membranes have provided evidence of possible MOAs. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-

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[4] Bio/dynamics, Inc. 1992. A two week inhalation toxicity study of C-437 and C-1754 (ethoxylated para-tertiary-octyl phenol) in the rat with cover letter dated 5/24/96 (sanitized). NTIS Report No. OTS0573048.

dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg et al (2019) evaluated the cytotoxic activity of the of three nonionic polymeric surfactants, which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween (80) and Poloxamer 188in a BEAS-2B human bronchial epithelial cell model by using an innovative air-liquid interface (ALI) method of exposure compared to classical liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH) which is an intercellular enzyme present in large amounts in the cytoplasm. Loss of membrane integrity will cause the release of LDH into the extracellular medium. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method by measuring Lactate Dehydrogenase (LDH) activity, however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any in vitro cytotoxicity.

Altogether, the available in vitro and in vivo data indicate a wide discrepancy in respiratory toxicity among nonionic surfactants. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties such as surface tension or CMC. Others have examined the relationship between chemical properties of nonionic surfactants and eye irritation and concluded that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths failed to predict eye irritation potential across the nonionic subcategory (Heinze et al., 1999). However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when conducted under nonequilibrium conditions. Whether this chemical property similarly predicts potency of nonionic surfactants to induce respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

Two acute inhalation toxicity studies were identified for several anionic surfactants which demonstrated high toxicity via the inhalation route. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. All 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. Animals in the 0.05 mg/l had no clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Commented [OS26]: Mike/Wayne have indicated that this does not meet the boundary criteria. It is quite insoluble, etc. More information to follow.

Commented [OS27R26]: William will address this in the table re: oleoyl sarcosine and sodium salt version.

Repeated-dose inhalation studies were identified for oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[5]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 µm and the geometric standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Commented [ST28]: Todd will add these footnotes (i.e., 3 + 4) to EndNote file

Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[6]. There were no statistically significant differences in dosed and control

[5] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

[6] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity,

groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Mechanistic studies examining the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

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6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four
4-day mini-cumulative irritation. Submission of unpublished data by CTFA,
200 pp.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. For example, inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, 1.36 mg/L, or 4.54 mg/L for 2 hours observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 μm and the geometric standard deviation (GSD) was 2.75 μm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 μm and the GSD was 1.83-1.86 μm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 µm, and the GSD was 1.62-1.65 µm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 µm and the GSD was 1.51 to 2.00 µm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC has greater deposition to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole which were observed in the study were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Mechanistic studies

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels demonstrated significant differences when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

Commented [HT29]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For

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HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (EPA, 2019). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 because ADME differences that would otherwise influence toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

Commented [HT31]: Need Citation at end of this paragraph...assume it is the OPP Guidance??

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosimetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST32]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEl9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or

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decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST34]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

Commented [HT35R34]: Tell us how? Describe the RELATIONSHIP, E.G. increase in ST would increase/decrease toxicity and increase/decrease in CMC is correlated with increases/decreases tox...are there papers on these relationships?

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in* *vitro* irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, $\leq$ 10 µm). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

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In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

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In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
			• Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells • MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

Commented [ST39]: William comment: "Corrosion can be due to acidity, alkalinity or the inherent ability to cause cellular necrosis. Alkaline or acidic compounds can be pH adjusted to neutral values."

Commented [ST40]: Todd to add to EndNote file

CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Commented [ST41]: Note, the SmallAir system should be added to the above table, as possible OLE test systems

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA42]: Issue Paper
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MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as a co-culture of cells from small airways (SmallAir™). EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system ~~with normal human stromal fibroblasts,~~ and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a

positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019).

Commented [RAB43]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB44]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be monitored. One further advantage of PCLS is that the PCLS assay can be performed on multiple species to determine susceptibility.

Commented [SM45]: **Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices**
Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP,

a priori to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB46]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230015000392"]
[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

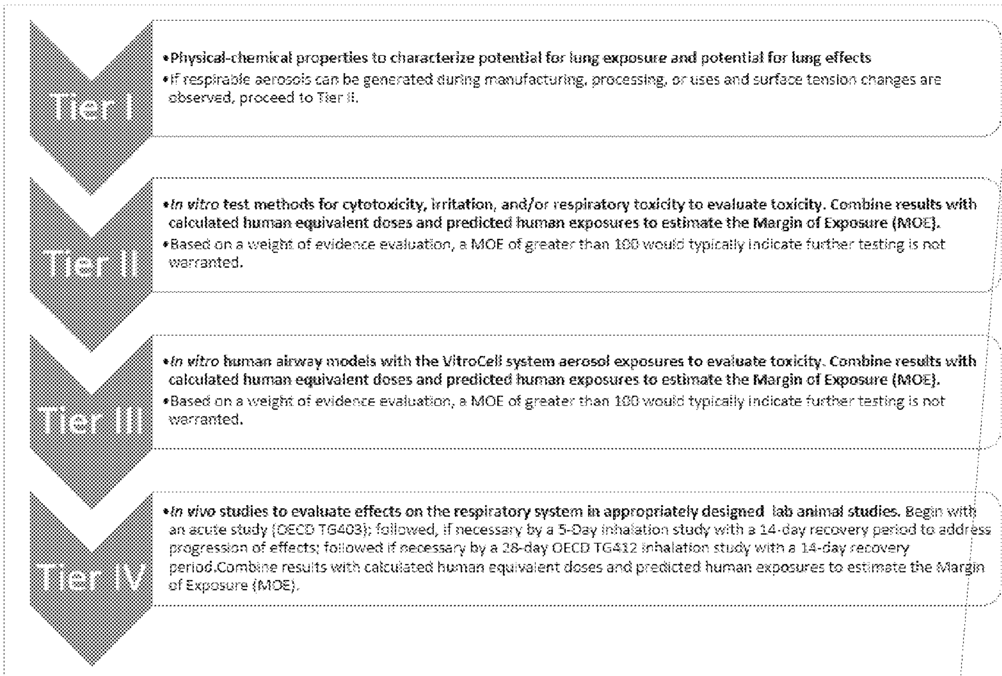
Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Commented [OS47]: Tala to include some additional text – read across, etc.

Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.



Commented [OS48]: Additional changes coming

Tier I—Physical-chemical properties

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If respirable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Commented [OS49]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST50R49]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information ~~towards~~ with informing MIEs and CLEs. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA should be considered, given that none of the following studies are validated to determine lung toxicity induced by surfactants. In general, the testing approach should include a combination of assays, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro*/*ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Pulmonary surfactant interaction/loss of function

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

General cytotoxicity

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of the assays may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified from the assays should be used to calculate a POD using BMD modeling, when possible, with the $BMCL_{1SD}$ metric. This metric is based on the benchmark response (BMR) of one standard

deviation suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). ~~Regardless of the metric used, a justification for its selection should be provided. The *in vitro* POD can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols.~~ In those situations where data are not amenable to BMD modeling, due to assays that are not designed to provide concentration response data and/or lack sufficient granularity, the *in vitro* testing concentration level should be determined based on the expected HEC (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in vitro* testing exposure dose.

Commented [ST51]: Note, I deleted this b/c of the statement above about using RDDR or MPPD for determining test concentrations.

When the data are amenable to calculating an HEC, the relevant routes of exposure should be considered, based on the conditions of use. An ~~margin of exposure~~MOE may then be determined by dividing the HEC by the estimated exposure ~~and comparing to the benchmark MOE for the respective positive control~~.

Commented [RAB52]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the 3 assays, will drive the determination of "greater" or

Commented [RAB53]: Its not clear how MOE fits into these decision criteria. I inserted draft text below – highlighted -- as a suggestion – please review and revise as needed

"comparable" toxicity, whereas negative results in all 3 assays will drive the determination of "lower" toxicity, as described below.

If the new chemical substance exhibits greater toxicity to the positive control in one of the evaluated assays, per the study method criteria, proceed to Tier III.

If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the ~~margin of exposures~~MOE for specific conditions of manufacturing, formulation, and use to consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then recalculate the MOE ~~reassess risks~~ using the modified POD_{HEC}. ~~using MOE as the risk metric~~ If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

Tier III – Human Airway Models/PCLS Assay

- ~~Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures~~

~~*In vitro* to *in vivo* extrapolation to develop a $_{HEC}$ in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the $_{HEC}$ by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the $_{HEC}$ can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these substances leading to impacts on lung surfactant or cell membranes. Because these effects are related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf).~~

Commented [KA54]: Issue Paper
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August 30, 2018

Commented [OS55]: Stay consistent AOP not MoA – search throughout

Commented [ST56R55]: I deleted this because it seems redundant with the Category benchmark MOE discussion.

Commented [ST57]: I deleted this because it doesn't appear relevant to our situation. The ECETOC document specifies that the reduction to unity is for gases and vapors, not aerosols. See p. 29 of the cited document.

Several testing options are available for evaluating OLEs in the surfactant AOP. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (e.g., TB and/or PU regions) using RDDR or MPPD modeling, as discussed previously. A justification for using a particular system(s) versus another should be provided and may be discussed with EPA as part of a pre-notice consultation. Available test systems include, but are not limited to, the following:

- EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- Precision-cut lung slice test *etc.* as described by Hess et al (2016)

Commented [ST58]: Note, the SmallAir system should be included here

Based on the results of the 3D-construct and/or PCLS testing, *in vitro* to *in vivo* extrapolation may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

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If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use, then consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the Tier III test data are not amenable for developing a POD_{IVIVC}, then proceed to Tier IV.

~~A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (e.g., engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.~~

Tier IV—*In vivo* studies

Strategic *in vivo* testing may be needed to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the subcategory analogues is appropriate for read across, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving POD_{IVIVC}s. If *in vivo* testing is needed, a pre-notice consultation meeting with EPA should be considered prior to initiating any testing.

~~Note that a prenotification consultation with EPA should be considered prior to undertaking any Tier IV testing.~~

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with pulmonary surfactant and/or disrupting cellular membranes. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests are recommended:

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. ~~As described above, the HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is > 100, further testing is not needed.~~

- Step 2: 5-Day inhalation study with a 14-day recovery period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). ~~Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is > 100, further testing is not needed.~~

- ~~Step 3. OECD TG 412**: 28-day inhalation study in rats with a 14-day recovery period.~~

Commented [ST60]: Recommend deleting, if there are concerns for effects in the respiratory tract consistent with the surfactant AOP, they will show up in the 5-day inhalation study.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA61]: Alarie, Y., G.D. Nielsen, and M.M. Sch bioassays for evaluation of indoor air quality *Quality Handbook*. Spengler, J.D., J.M J.F. McCarthy (eds.). New York: McGra pp 23.21-23.49.

The results of the *in vivo* testing may be used for reassessing and recharacterizing the previously identified risks under the conditions of use for the new chemical substance. Depending on the outcome of the risk assessment, EPA will apply risk management actions on those conditions of use that result in findings of unreasonable risk, whereas no restrictions would be applied on the conditions of use where the MOEs exceed the benchmark MOE.

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

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Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Message

From: Stedeford, Todd [Stedeford.Todd@epa.gov]
Sent: 7/24/2020 10:43:28 AM
To: Sahar_Osman-Sypher@americanchemistry.com
CC: Henry, Tala [Henry.Tala@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]
Subject: RE: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure
Attachments: draft manscript general surfactants - 23 July 2020.ver.3.docx

Here is a revised draft Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

From: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Sent: Thursday, July 23, 2020 12:03 PM
To: Stedeford, Todd <Stedeford.Todd@epa.gov>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure
Importance: High

Todd:

Attached is the latest version of the manuscript (July 23, Version 2) with discussions from the call incorporated. I've also added the updated tables and tiered testing figure.

Regards, Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

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KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc*.), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate, a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol, a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS2]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled " ".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled " ".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	CASRN
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of

this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB3]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT4]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA has, for decades, relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT5]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

1 Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.*, a dose or concentration that marks the beginning of a low-dose

Commented [HT6]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Exposure Assessment

Commented [HT7]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments…include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value is 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

Commented [HT8]: (U.S. EPA, 1994).

Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most

often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

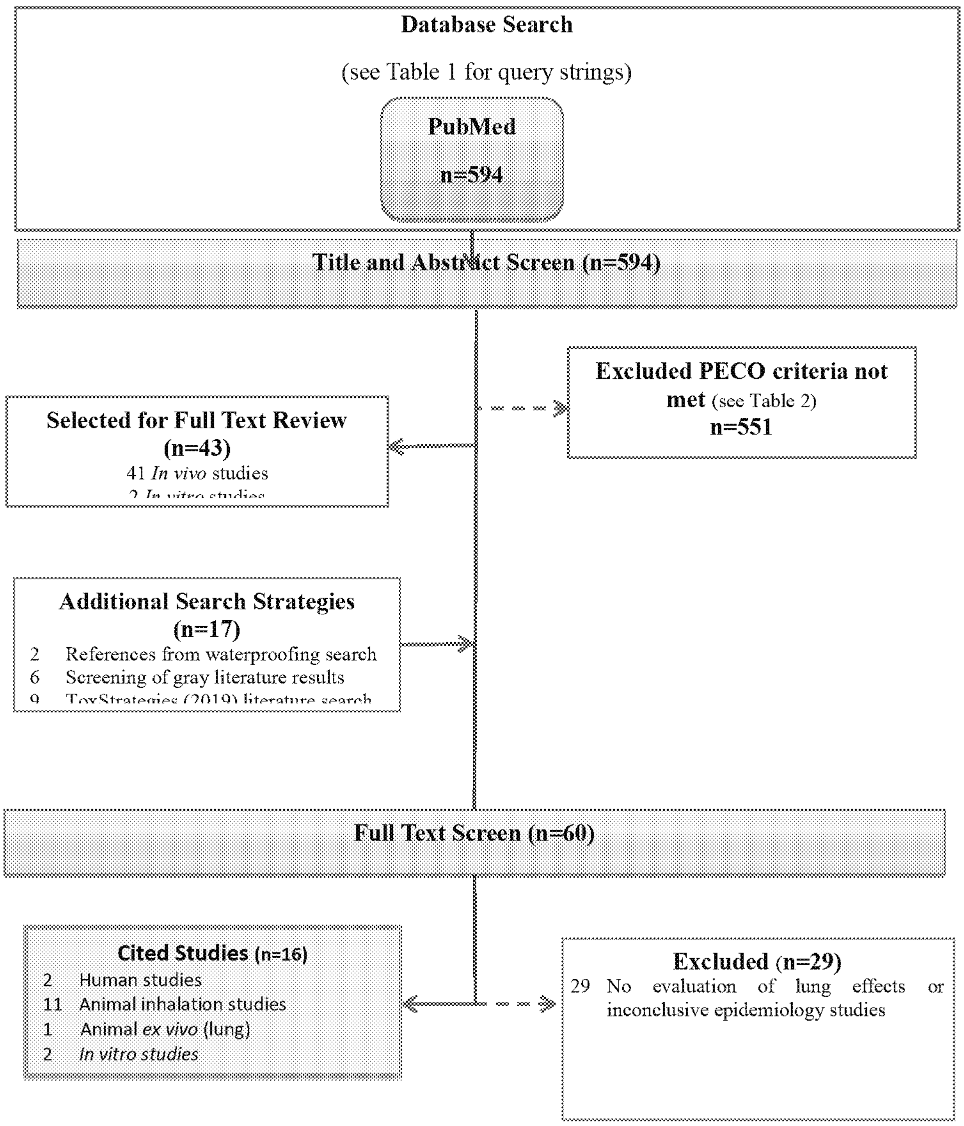
The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

Commented [ST9]: This section needs updating following final disposition of gray lit and Updated Literature Search.

Scheme 1. Literature search and screening flow diagram for surfactants

Commented [ST10]: The tally of Cited and Excluded references from the bottom of the figure includes the PubMed results only. These boxes need to be updated following disposition of 6 studies from the gray lit. search and 9 studies from the Updated Literature Search.



Category Boundaries

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

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[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and the assessment should be conducted on the predominant ~~or both~~ components. The non-ionized fraction for acids/bases should be calculated as follows.

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{pH-pKa})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{pKa-pH})$$

Where the pH represents the physiological pH in the lung (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine). ~~A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.~~

Commented [KA11]: Should this sentence be deleted?

Commented [OS12]: Todd will update to simplify, show equation,
Aromatic amines is an aniline, ph7

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

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Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Commented [ST17]: Not in Mike's Table

Commented [ST18]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"]
this paper shows ST to be a lot higher

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

Commented [ST19]: Concentration?

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[INSERT TABLE X]

Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Nonionic Surfactants

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed

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Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS23]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

Commented [OS24]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

In rodent models, irritation and inflammatory effects on the respiratory tract has been observed with varying degrees of severity. Acute inhalation exposure to Polysorbate 20 via nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/l (5,100 mg/m^3) did not observed in mortalities, clinical signs, or abnormalities in the gross pathology[3]. Using MPPD modeling, the total lung deposition mass was calculated to be 6.6E+4 µg. A respiratory irritation study was conducted on a mixture containing Nonidet in male Webster mice using the ASTM Method E981 where animals were exposed for 3 hours to concentrations of 12, 22, 51, 118, and

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[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3"]

134 mg/m^3 (Alarie and Stock, 1992, unpublished). Signs of respiratory irritation was observed in animals at the three highest concentrations as indicated by increased respiratory frequency without an increase in pulmonary edema or lung weight. An acute inhalation exposure study in Syrian hamsters to 3.0 mg/l of Triton X-100 to varying exposure durations reported that lung deposition of Triton X-100 corresponded to mortality with an LD50 of 1300-2100 µg (Damon et al., 1982). The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways and lungs in these animals were relatively free of serious pathologies. The authors hypothesized that that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the deposited chemical resulted in a large concentration of the chemical on the laryngeal mucosa. Finally, in the only repeated dose inhalation exposure identified for nonionic surfactants, a 2-week repeated dose inhalation study was conducted on Triton X-100 in male and female Sprague-Dawley rats to 5.3 mg/m^3 (MMAD 1.8 µm, GSD 1.8µm) for 6 hours/day, 5 days/week (Bio/dynamics, Inc. 1992[4].) Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge.

Commented [SK25]: It is unclear to me if the other tested concentration should be included since it is a 70% mixture.

In vitro studies of surfactant effects on cell membranes have provided evidence of possible MOAs. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-

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[4] Bio/dynamics, Inc. 1992. A two week inhalation toxicity study of C-437 and C-1754 (ethoxylated para-tertiary-octyl phenol) in the rat with cover letter dated 5/24/96 (sanitized). NTIS Report No. OTS0573048.

dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg et al (2019) evaluated the cytotoxic activity of the of three nonionic polymeric surfactants, which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween (80) and Poloxamer 188in a BEAS-2B human bronchial epithelial cell model by using an innovative air-liquid interface (ALI) method of exposure compared to classical liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH) which is an intercellular enzyme present in large amounts in the cytoplasm. Loss of membrane integrity will cause the release of LDH into the extracellular medium. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method by measuring Lactate Dehydrogenase (LDH) activity, however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any in vitro cytotoxicity.

Altogether, the available in vitro and in vivo data indicate a wide discrepancy in respiratory toxicity among nonionic surfactants. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties such as surface tension or CMC. Others have examined the relationship between chemical properties of nonionic surfactants and eye irritation and concluded that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths failed to predict eye irritation potential across the nonionic subcategory (Heinze et al., 1999). However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when conducted under nonequilibrium conditions. Whether this chemical property similarly predicts potency of nonionic surfactants to induce respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

Two acute inhalation toxicity studies were identified for several anionic surfactants which demonstrated high toxicity via the inhalation route. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. All 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. Animals in the 0.05 mg/l had no clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

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Repeated-dose inhalation studies were identified for oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[5]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 μm and the geometric standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

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Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[6]. There were no statistically significant differences in dosed and control

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[5] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

[6] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity,

groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Mechanistic studies examining the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

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6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four
4-day mini-cumulative irritation. Submission of unpublished data by CTFA,
200 pp.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. For example, inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, 1.36 mg/L, or 4.54 mg/L for 2 hours observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 μm and the geometric standard deviation (GSD) was 2.75 μm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 μm and the GSD was 1.83-1.86 μm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 μm, and the GSD was 1.62-1.65 μm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 μm and the GSD was 1.51 to 2.00 μm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC has greater deposition to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole which were observed in the study were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Mechanistic studies

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 μg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels demonstrated significant differences when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For

Commented [HT29]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

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HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (EPA, 2019). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 because ADME differences that would otherwise influence toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

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1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosimetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST32]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEl9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or

Commented [HT33]: This is where WI's comment re: predicting potency, if turned into actual explanation with citations, could be useful.

decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST34]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

Commented [HT35R34]: Tell us how? Describe the RELATIONSHIP, E.G. increase in ST would increase/decrease toxicity and increase/decrease in CMC is correlated with increases/decreases tox...are there papers on these relationships?

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in* *vitro* irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, ≤ 10 μm). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

Commented [KA36]: Arch Toxicol . 2017 Nov;91(11):3477-3505. doi: 10.1007/s00204-017-2045-3.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

Commented [KA37]: I am not sure what is meant here. Needs rewording by author.

Commented [SK38]: This belongs in the NAMs section not hazard ID. I've moved.

In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
			• Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells • MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

Commented [ST39]: William comment: "Corrosion can be due to acidity, alkalinity or the inherent ability to cause cellular necrosis. Alkaline or acidic compounds can be pH adjusted to neutral values."

Commented [ST40]: Todd to add to EndNote file

CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Commented [ST41]: Note, the SmallAir system should be added to the above table, as possible OLE test systems

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA42]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as cells from small airways. EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system with normal human stromal fibroblasts, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Commented [OS43]: Scott Slattery Comment: This is a separate product from Epithelix called SmallAir.

Commented [OS44]: Scott Slattery Comment: This is a distinct product called EpiAirwayFT. The standard EpiAirway does not contain stromal fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a

positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019) .

Commented [RAB45]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB46]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be monitored. One further advantage of PCLS is that the PCLS assay can be performed on multiple species to determine susceptibility.

Commented [SM47]: **Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices**
Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP,

a priori to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB48]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230015000392"]
[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

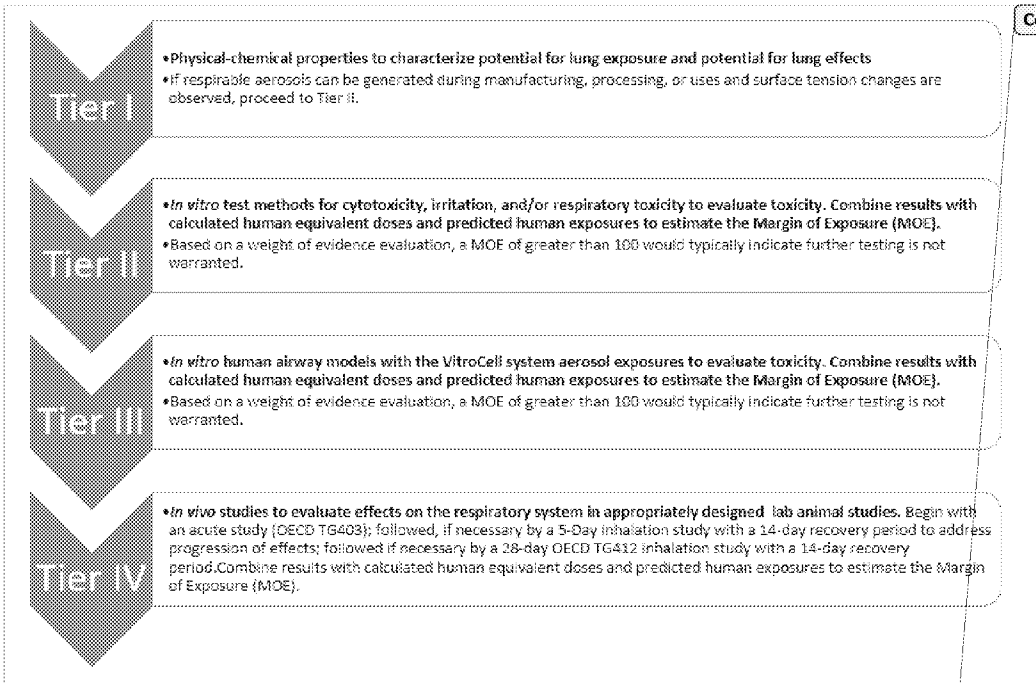
Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Commented [OS49]: Tala to include some additional text – read across, etc.

Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.



Commented [OS50]: Additional changes coming

Tier I—Physical-chemical properties

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If respirable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Commented [OS51]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST52R51]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information ~~towards~~ with informing MIEs and CLEs. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA should be considered, given that none of the following studies are validated to determine lung toxicity induced by surfactants. In general, the testing approach should include a combination of assays, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro*/*ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Pulmonary surfactant interaction/loss of function

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

General cytotoxicity

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of the assays may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified from the assays should be used to calculate a POD using BMD modeling, when possible, with the $BMCL_{1SD}$ metric. This metric is based on the benchmark response (BMR) of one standard

deviation suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). Regardless of the metric used, a justification for its selection should be provided. ~~The *in vitro* POD can be converted to a deposited dose using the Multiple Path Particle Dosimetry (MPPD) model for aerosols.~~ In those situations where data are not amenable to BMD modeling, due to assays that are not designed to provide concentration response data and/or lack sufficient granularity, the *in vitro* testing concentration level should be determined based on the expected HEC (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in vitro* testing exposure dose.

Commented [ST53]: Note, I deleted this b/c of the statement above about using RDDR or MPPD for determining test concentrations.

When the data are amenable to calculating an HEC, the relevant routes of exposure should be considered, based on the conditions of use. An ~~margin of exposure~~MOE may then be determined by dividing the HEC by the estimated exposure and comparing to the benchmark MOE for the respective positive control.

Commented [RAB54]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the 3 assays, will drive the determination of "greater" or

Commented [RAB55]: Its not clear how MOE fits into these decision criteria. I inserted draft text below – highlighted -- as a suggestion – please review and revise as needed

"comparable" toxicity, whereas negative results in all 3 assays will drive the determination of "lower" toxicity, as described below.

If the new chemical substance exhibits greater toxicity to the positive control in one of the evaluated assays, per the study method criteria, proceed to Tier III.

If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the ~~margin of exposures~~MOE for specific conditions of manufacturing, formulation, and use to consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then recalculate the MOE ~~reassess risks~~ using the modified POD_{HEC}. ~~using MOE as the risk metric~~ If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

Tier III – Human Airway Models/PCLS Assay

- ~~Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures~~

~~*In vitro* to *in vivo* extrapolation to develop a HEC in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the HEC by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the HEC can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these substances leading to impacts on lung surfactant or cell membranes. Because these effects are related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf).~~

Several testing options are available for evaluating OLEs in the surfactant AOP. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (*e.g.*, TB and/or PU regions) using RDDR or MPPD modeling, as discussed previously. A justification for using a particular system(s) versus another should be provided and may be discussed with EPA as part of a pre-notice consultation. Available test systems include, but are not limited to, the following.

Commented [KA56]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

Commented [OS57]: Stay consistent AOP not MoA – search throughout

Commented [ST58R57]: I deleted this because it seems redundant with the Category benchmark MOE discussion.

Commented [ST59]: I deleted this because it doesn't appear relevant to our situation. The ECETOC document specifies that the reduction to unity is for gases and vapors, not aerosols. See p. 29 of the cited document.

- EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- Precision-cut lung slice test *etc.* as described by Hess et al (2016)

Commented [ST60]: Note, the SmallAir system should be included here

Based on the results of the 3D-construct and/or PCLS testing, *in vitro* to *in vivo* extrapolation may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

Commented [KA61]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use, then consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

~~A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (e.g., engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.~~

Tier IV—*In vivo* studies

Strategic *in vivo* testing may be needed to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the subcategory analogues is appropriate for read across, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving POD_{HEC}s. If *in vivo* testing is needed, a pre-notice consultation meeting with EPA should be considered prior to initiating any testing.

~~Note that a prenotification consultation with EPA should be considered prior to undertaking any Tier IV testing.~~

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with pulmonary surfactant and/or disrupting cellular membranes. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests are recommended:

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. ~~As described above, the HEC should be derived using default or chemical-specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is > 100, further testing is not needed.~~

- Step 2: 5-Day inhalation study with a 14-day recovery period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). ~~Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical-specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is > 100, further testing is not needed.~~

- ~~Step 3. OECD TG 412**: 28-day inhalation study in rats with a 14-day recovery period.~~

Commented [ST62]: Recommend deleting, if there are concerns for effects in the respiratory tract consistent with the surfactant AOP, they will show up in the 5-day inhalation study.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA63]: Alarie, Y., G.D. Nielsen, and M.M. Sch bioassays for evaluation of indoor air quality *Quality Handbook*. Spengler, J.D., J.M J.F. McCarthy (eds.). New York: McGra pp 23.21-23.49.

The results of the *in vivo* testing may be used for reassessing and recharacterizing the previously identified risks under the conditions of use for the new chemical substance. Depending on the outcome of the risk assessment, EPA will apply risk management actions on those conditions of use that result in findings of unreasonable risk, whereas no restrictions would be applied on the conditions of use where the MOEs exceed the benchmark MOE.

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

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Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Message

From: Osman-Sypher, Sahar [Sahar_Osman-Sypher@americanchemistry.com]
Sent: 7/23/2020 4:03:02 PM
To: Stedeford, Todd [Stedeford.Todd@epa.gov]
CC: Henry, Tala [Henry.Tala@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]
Subject: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure
Attachments: draft manscript general surfactants - 23 July 2020.ver.2.docx; Table X Example Surfactants in Subcategories_07-23-20.docx; Table Y Haz ID and D-R Table_07-23-20.docx; Tiered Testing Figure rev 23 July 2020.pptm

Importance: High

Todd:

Attached is the latest version of the manuscript (July 23, Version 2) with discussions from the call incorporated. I've also added the updated tables and tiered testing figure.

Regards, Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

Tala R. Henry[a,‡], Keith Salazar[b,‡], Michael P. Hayes[c], Wayne Kennedy[d], Athena M. Keene[d], Annie Jarabek[e], Stefan Moors[f], Lela Jovanovich[g], Raphael Tremblay[c], Ann Tveit[f], Richard A. Becker[h], Sahar Osman-Sypher[h], Patrick D. McMullen[i], Scott D. Slattery[i], William Irwin[b], Marc Odin[j], and Todd Stedeford[a,]*

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KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc*.), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate, a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol, a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS2]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled "".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled "".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of

this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB3]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT4]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA has, for decades, relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT5]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

1 Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.,* a dose or concentration that marks the beginning of a low-dose

Commented [HT6]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Exposure Assessment

Commented [HT7]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments…include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value is 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	$Cm \times b \times h$, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: $Cm = KCk \times Ys/Ypel$

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

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Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most

often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

Commented [ST9]: This section needs updating following final disposition of gray lit and Updated Literature Search.

Scheme 1. Literature search and screening flow diagram for surfactants

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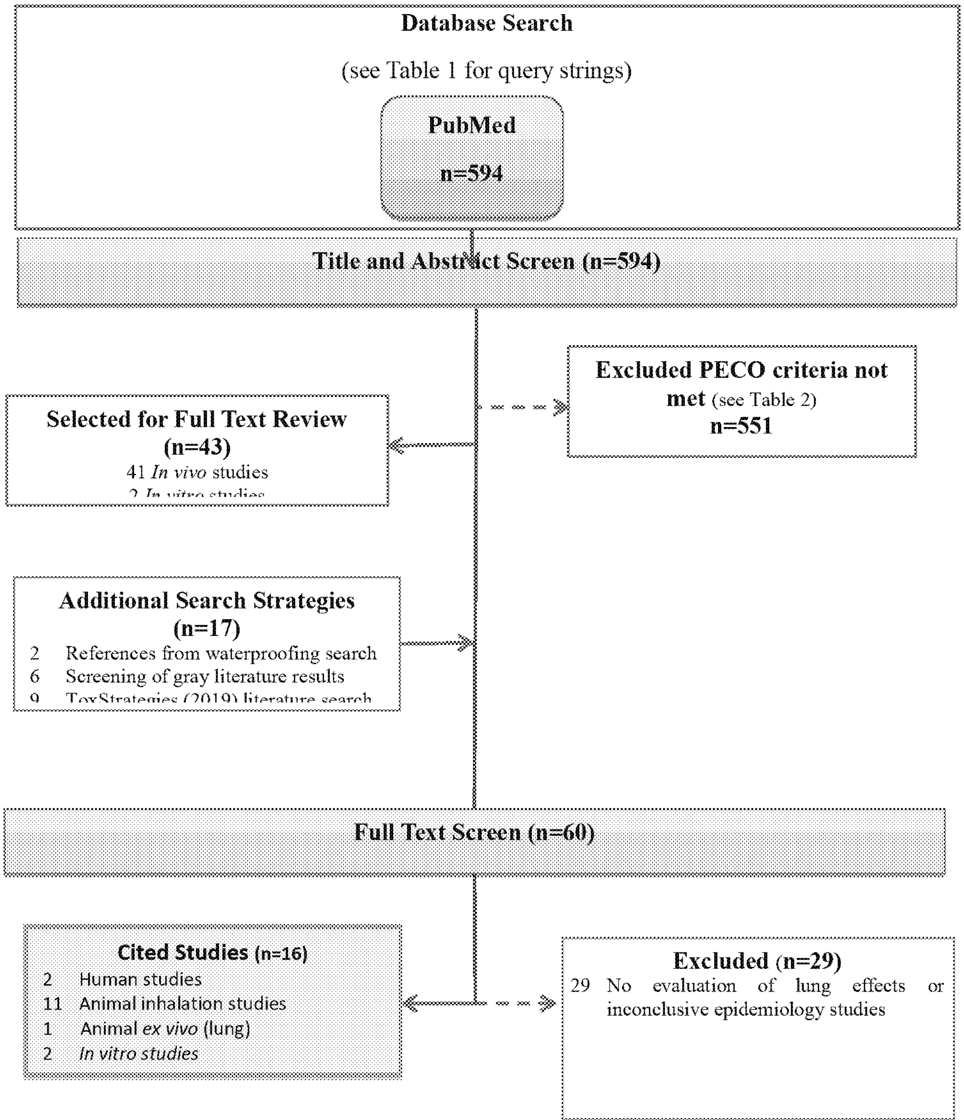

Category Boundaries

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

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[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and the assessment should be conducted on the predominant or both components. A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.

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Commented [OS12]: Todd will update to simplify, show equation,
Aromatic amines is an aniline, ph7

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

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Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

[INSERT TABLE X]

Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in

Commented [ST17]: Not in Mike's Table

Commented [ST18]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"]
this paper shows ST to be a lot higher

Commented [ST19]: Concentration?

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Commented [ST22]: Add the following, based on Updated Literature Search?
Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS23]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Nonionic Surfactants

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Commented [OS24]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985;

Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

In rodent models, irritation and inflammatory effects on the respiratory tract has been observed with varying degrees of severity. Acute inhalation exposure to Polysorbate 20 via nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/l (5,100 mg/m^3) did not observed in mortalities, clinical signs, or abnormalities in the gross pathology[3]. Using MPPD modeling, the total lung deposition mass was calculated to be 6.6E+4 μg. A respiratory irritation study was conducted on a mixture containing Nonidet in male Webster mice using the ASTM Method E981 where animals were exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m^3 (Alarie and Stock, 1992, unpublished). Signs of respiratory irritation was observed in animals at the three highest concentrations as indicated by increased respiratory frequency without an increase in pulmonary edema or lung weight. An acute inhalation exposure study in Syrian hamsters to 3.0 mg/l of Triton X-100 to varying exposure durations reported that lung deposition of Triton X-100 corresponded to mortality with an LD50 of 1300-2100 μg (Damon et al., 1982). The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways and lungs in these animals were relatively free of serious pathologies. The authors hypothesized that that these observed effects were due to

[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3"]

large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the deposited chemical resulted in a large concentration of the chemical on the laryngeal mucosa. Finally, in the only repeated dose inhalation exposure identified for nonionic surfactants, a 2-week repeated dose inhalation study was conducted on Triton X-100 in male and female Sprague-Dawley rats to 5.3 mg/m^3 (MMAD 1.8 µm, GSD 1.8µm) for 6 hours/day, 5 days/week (Bio/dynamics, Inc. 1992[4].) Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge.

Commented [SK25]: It is unclear to me if the other tested concentration should be included since it is a 70% mixture.

In vitro studies of surfactant effects on cell membranes have provided evidence of possible MOAs. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

[4] Bio/dynamics, Inc. 1992. A two week inhalation toxicity study of C-437 and C-1754 (ethoxylated para-tertiary-octyl phenol) in the rat with cover letter dated 5/24/96 (sanitized). NTIS Report No. OTS0573048.

Lindenberg et al (2019) evaluated the cytotoxic activity of the of three nonionic polymeric surfactants, which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween (80) and Poloxamer 188in a BEAS-2B human bronchial epithelial cell model by using an innovative air-liquid interface (ALI) method of exposure compared to classical liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH) which is an intercellular enzyme present in large amounts in the cytoplasm. Loss of membrane integrity will cause the release of LDH into the extracellular medium. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method by measuring Lactate Dehydrogenase (LDH) activity, however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any in vitro cytotoxicity.

Altogether, the available in vitro and in vivo data indicate a wide discrepancy in respiratory toxicity among nonionic surfactants. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties such as surface tension or CMC. Others have examined the relationship between chemical properties of nonionic surfactants and eye irritation and concluded that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths failed to predict eye irritation potential across the nonionic subcategory (Heinze et al., 1999). However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when conducted under nonequilibrium conditions. Whether this chemical property similarly predicts potency of nonionic surfactants to induce respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

Two acute inhalation toxicity studies were identified for several anionic surfactants which demonstrated high toxicity via the inhalation route. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. All 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. Animals in the 0.05 mg/l had no clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Commented [OS26]: Mike/Wayne have indicated that this does not meet the boundary criteria. It is quite insoluble, etc. More information to follow.

Commented [OS27R26]: William will address this in the table re: oleoyl sarcosine and sodium salt version.

Repeated-dose inhalation studies were identified for oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[5]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 μm and the geometric

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[5] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[6]. There were no statistically significant differences in dosed and control groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red

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[6] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity, 6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four 4-day mini-cumulative irritation. Submission of unpublished data by CTFA, 200 pp.

O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Mechanistic studies examining the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. For example, inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, 1.36 mg/L, or 4.54 mg/L for 2 hours observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 μm and the geometric standard deviation (GSD) was 2.75 μm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 µm and the GSD was 1.83-1.86 µm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 µm, and the GSD was 1.62-1.65 µm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 μm and the GSD was 1.51 to 2.00 μm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC has greater deposition to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole which were observed in the study were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Mechanistic studies

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels demonstrated significant differences when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

Commented [HT29]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

Commented [HT30]: Maybe this goes in the TSCA methods section? Or, because its 'new' to this category, put here?

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference*

Concentrations and Application of Inhalation Dosimetry" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are

Commented [HT31]: Need Citation at end of this paragraph...assume it is the OPP Guidance??

reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 because ADME differences that would otherwise influence toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosimetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

$UF_H = 10$ or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST32]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEI9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

Commented [HT33]: This is where WI's comment re: predicting potency, if turned into actual explanation with citations, could be useful.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST34]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

Commented [HT35R34]: Tell us how? Describe the RELATIONSHIP, E.G. increase in ST would increase/decrease toxicity and increase/decrease in CMC is correlated with increases/decreases tox…are there papers on these relationships?

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, ≤ 10 μm). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant

Commented [KA36]: Arch Toxicol . 2017 Nov;91(11):3477-3505. doi: 10.1007/s00204-017-2045-3.

(MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

Commented [KA37]: I am not sure what is meant here. Needs rewording by author.

Commented [SK38]: This belongs in the NAMs section not hazard ID. I've moved.

In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
			• Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells • MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

Commented [ST39]: William comment: "Corrosion can be due to acidity, alkalinity or the inherent ability to cause cellular necrosis. Alkaline or acidic compounds can be pH adjusted to neutral values."

Commented [ST40]: Todd to add to EndNote file

CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA41]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as cells from small airways. EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system with normal human stromal fibroblasts, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Commented [OS42]: Scott Slattery Comment: This is a separate product from Epithelix called SmallAir.

Commented [OS43]: Scott Slattery Comment: This is a distinct product called EpiAirwayFT. The standard EpiAirway does not contain stromal fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a

positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019).

Commented [RAB44]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB45]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be monitored. One further advantage of PCLS is that the PCLS assay can be performed on multiple species to determine susceptibility.

Commented [SM46]: **Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices**
Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP,

a priori to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB47]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230015000392"]
[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

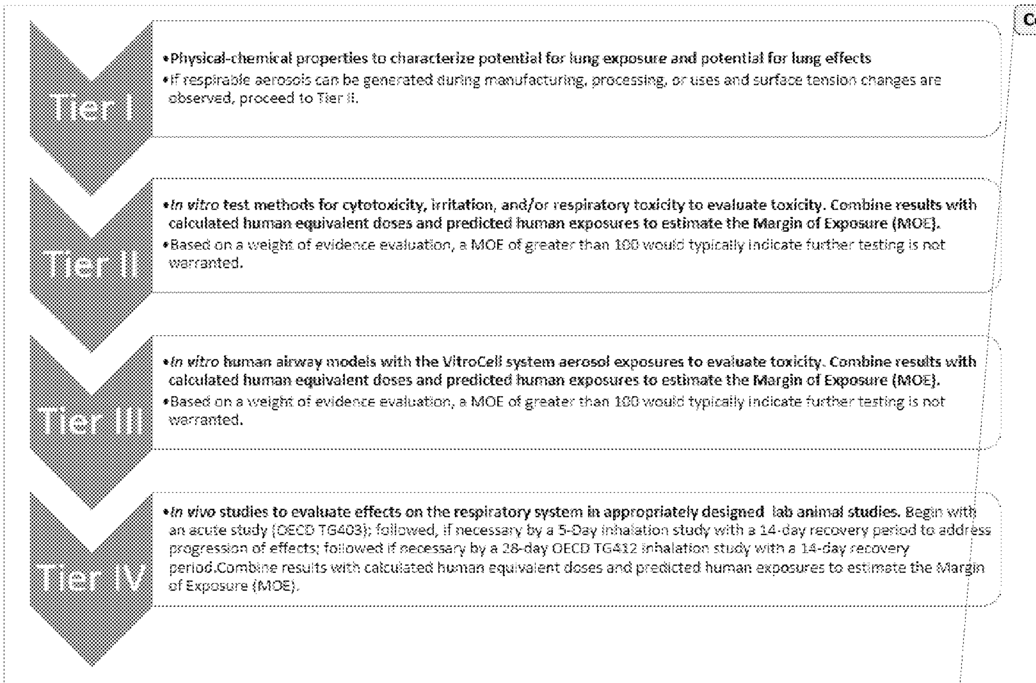
Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Commented [OS48]: Tala to include some additional text – read across, etc.

Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.



Commented [OS49]: Additional changes coming

Tier I—Physical-chemical properties

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If respirable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Commented [OS50]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST51R50]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information towards informing MIEs and CLEs. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA should be considered, given that none of the following studies are validated to determine lung toxicity. induced by surfactants. In general, the testing approach should include a combination of assays, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro*/*ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Pulmonary surfactant interaction/loss of function

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

General cytotoxicity

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of the assays may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified from the assays should be used to calculate a POD using BMD modeling, when possible, with the $BMCL_{1SD}$ metric. This metric is based on the benchmark response (BMR) of one standard

deviation suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). The *in vitro* POD can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols. In those situations where data are not amenable to BMD modeling, due to assays that are not designed to provide concentration response data and/or lack sufficient granularity, the *in vitro* testing concentration level should be determined based on the expected HEC (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in vitro* testing exposure dose.

When the data are amenable to calculating an HEC, the relevant routes of exposure should be considered, based on the conditions of use. A margin of exposure may then be determined by dividing the HEC by the estimated exposure.

Commented [RAB52]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the 3 assays, will drive the determination of "greater" or "comparable" toxicity, whereas negative results in all 3 assays will drive the determination of "lower" toxicity, as described below.

Commented [RAB53]: Its not clear how MOE fits into these decision criteria. I inserted draft text below – highlighted -- as a suggestion – please review and revise as needed

If the new chemical substance exhibits greater toxicity to the positive control in one of the evaluated assays, per the study method criteria, proceed to Tier III.

If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the margin of exposures for specific conditions of manufacturing, formulation and use to consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then reassess risks using the modified POD_{HEC}. using MOE as the risk metric If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

Tier III – Human Airway Models/PCLS Assay

- Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures

In vitro to *in vivo* extrapolation to develop a HEC in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the HEC by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the HEC can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these substances leading to impacts on lung surfactant or cell membranes. Because these effects are related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf). A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (*e.g.*, engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.

Commented [KA54]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

Commented [OS55]: Stay consistent AOP not MoA – search throughout

Tier IV—*In vivo* studies

Note that a prenotification consultation with EPA should be considered prior to undertaking any Tier IV testing.

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. As described above, the HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is > 100, further testing is not needed.

- Step 2: 5-Day inhalation study with a 14-day recovery period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is > 100, further testing is not needed.

- Step 3: OECD TG 412**: 28-day inhalation study in rats with a 14-day recovery period.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA56]: Alarie, Y., G.D. Nielsen, and M.M. Sch bioassays for evaluation of indoor air quality *Quality Handbook*. Spengler, J.D., J.M J.F. McCarthy (eds.). New York: McGra pp 23.31–23.49.

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

ACKNOWLEDGMENT

Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Table X. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cation Subcategorization

Chemical Name in Text	Other Relevant Names	Criteria 1	Criteria 2	Criteria 3
Nonionic Surfactants				
Octoxynol 9 CASRN 9002-93-1	Triton X-100 Octylphenol ethoxylate 4-1,1,3,3-tetramethylbutylphenol ethoxylated ChemID*plus*: Octoxynol 9 ~~IUPAC: 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol~~ TSCA: Poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	Hydrophobic: octylphenol group Hydrophilic: polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C Reference: (1)	CMC is 0.17 or 0.017 wt% Reference: (1
~~Ethylene glycol n-hexyl ether (EGHE)~~ ~~CASRN 112-25-4~~	~~Ethylene glycol monohexyl ether~~ ~~ChemID*plus*: 2-hexyoxyethanol~~ ~~IUPAC: 2-(hexyloxy)ethan-1-ol~~ ~~TSCA: Ethanol, 2-(hexyloxy)-~~	~~Hydrophobic: hexoyl group~~ ~~Hydrophilic: ethyoxylate group~~	~~~33.5 mN/m at 10 g/L (1.0 wt%) and 25°C~~ ~~Reference: DOW?? from TEXT~~	
Tyloxapol Defomaire Alevaire CASRN 25301-02-4	Triton WR 1339 Formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol	Hydrophobic: multiple octyl phenol groups Hydrophilic: multiple	~37 mN/m at 5 g/L (0.5 wt%) and 25°C Reference: (1)	CMC is 0.038 g/L or 0.0038 wt% Reference: (1

Commented [HT1]: DONE:
1)Removed EGHE
2)Moved DDAO to Nonionic and put note about pH
3)Got full references

TO DO
1) Decide on 'other relvant names/ how many/which ones [im thinking ChemIDplus and TSCA...couldn't find IUPAC for all]
2) Cross walk Examples with TEXT and Studies having PODsClean up how Criteria 2 & 3 are presented; they vary throughout
3) DDAC criteria
4) SDS criteria
5) Format for citing the Refs in Table

Commented [HT3]: Verified names/synonyms and CASRN in ChemIDplus.
Provide ChemIDplus name as "reference"?
Provide ChemIDplus link? (could get old)
Should we streamline this table and put the details on names, etc in a supplemental?

References: can a table have its own references? OR do they have to be put into the overall Reference Section? (journal rules)

The Reference citations are incomplete; will need full cites. What is the Journal citation style?

Commented [HT2]: Went with using the "Chemical Name in Text" as the anchoring name, for transparency/crosswalk with the tox studies.
"other relevant names" are from ChemIDPlus (trade or common mostly from what was orginally provided...there can be A LOT of synonyms).
Put in IUPAC and TSCA names just to have a 'common denominator', but could not find for all...e.g., a couple of these chems appear NOT to be on TSCA Inventory

Commented [HT4]: Check spelling names say hexyloxy or hexyloxy; none have 'oyl'

Commented [HT5]: check

Commented [HT6]: No CMC

Commented [KW7]: Not a surfactant. Need to remove

Commented [HT8]: NOT in ChemIDplus as synonym

Commented [HT9]: NOT @ 20 deg...is there a 'conversion' method?

	ChemID*plus*: Tyloxapol TSCA: Not on TSCA Inventory	polyoxyethylene (9) units		
Polyoxyethylene-10-oleyl ether CASRN 9004-98-2	$C_{18:1}E_{10}$ Oleyl ethoxylate Oleth-10 Brij 97 ChemID*plus*: Polyoxyl 10 oleyl ether TSCA: Poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	Hydrophobic: oleyl group Hydrophilic: polyoxyethylene (10) unit	35.17 mN/m at CMC and 25°C from Table 1 ~37 mN/m at CMC and 25°C from Fig 1 Reference: (8)	$4x10^{-5}$ M at 25°C or 0.028 wt % Reference: (8)
Polyoxyethylene-10-dodecyl ether CASRN: 9002-92-0	$C_{12}E_{10}$ Polyethylene glycol monododecyl ether Polyoxyethylene (10) lauryl ether ChemID*plus*: Dodecyl alcohol, ethoxylated TSCA: Poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	Hydrophobic: dodecyl group Hydrophilic: polyoxyethylene (10) unit	C12E9: 36 mN/m at 23°C C12E12: 32 mN/m at 23°C Reference: (10)	$12.7x10^{-6}$ M at 30°C or 0.0008 wt % Reference (9) Also, C12E9 at $1x10^{-6}$ M at 23°C and C12E12 at $1.4X10^{-6}$ M at 23°C Reference: (10)
Polysorbate 20 Tween 20 CASRN 9005-64-5	Polyoxyethylene (20) sorbitan monolaurate ChemID*plus*: Polysorbate 20	Hydrophobic: dodecanoyl group Hydrophilic: sorbitan	38 mN/m at the CMC and 21°C Reference: (3)	$8.04x10^{-5}$ M at 21°C or 0.001 wt % Reference: (3)

Commented [HT10]: CehmID Plus shows Oleth **9; but not 10 as synonym**

	TSCA: Sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	polyoxyethylene (20) unit		
Polysorbate 80 Tween 80 CASRN 9005-65-6	Polyoxyethylene (20) sorbitan monooleate ChemID*plus*: Polysorbate 80 TSCA: Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	Hydrophobic: octadecenoyl group Hydrophilic: sorbitan polyoxyethylene (20) unit	37.96 mN/m at 0.5 wt % Reference: (5)	1.5×10^{-5} M at 25°C or 0.00[illegible] % Reference: (4)
Poloxamer 188 CASRN 691397-13-4	Pluronic F-68 ChemID*plus*: Poloxalene ~~IUPAC: 2-[2-(2-hydroxyethoxy)propoxy]ethanol~~ Oxirane, 2-methyl-, polymer with oxirane, triblock	Hydrophobic: polyoxypropylene (27) unit Hydrophilic: two polyoxyethylene (80) units	42-44 mN/m Reference: (6)	4.8×10^{-4} M at 37°C or 0.4 wt % Reference: (7)
N,N-Dimethyl-dodecylamine-N-oxide ($C_{12}AO$) CASRN 1643-20-5 Zwitterionic: At pH 7, 90% expected to be nonionic; only small amount cationic	1-Dodecanamine, *N,N*-dimethyl-, *N*-oxide Lauryl dimethylamine **oxide** ChemID*plus*: Lauramine oxide TSCA: 1-Dodecanamine, N,N-dimethyl-, N-oxide	Hydrophobic: dodecyl group Hydrophilic: amine oxide unit	32.6 mN/m at CMC Reference: (11)	1.7×10^{-3} M or 0.039 wt % Reference: (11) Mukerjee et al. also report values from 1×10^{-5} M to 5.5×10^{-5} M at 25°C Reference: (2 and 12)

Commented [HT11]: Temp//doesn't have g/L like others

Commented [HT12]: ChemIDplus indicates: N,N-Dimethyl-1-dodecanamine-N-oxide
And
N,N-Dimethyldodecylamine oxide (no 3rd N)

Anionic Surfactants				
Sodium dodecyl sulfate (SDS) CASRN: 151-21-3	ChemID*plus*: Sodium lauryl sulfate TSCA: Sulfuric acid monododecyl ester sodium salt (1:1)	Hydrophobic: ? Hydrophilic: ?	39.5 mN/m at 25°C from text Reference: ?	
Oleoyl sarcosine CASRN 110-25-8	ChemID*plus*: Oleoyl sarcosine TSCA: Glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-yl)- Acute Study Repeat-Dose Study	NO DATA HERE		
Sodium lauroyl sarcosinate CASRN: 137-16-6	Acute Study ChemID*plus*: Sodium lauroyl sarcosinate TSCA: Glycine, N-methyl-N-(1-oxododecyl)-, sodium salt (1:1)	NO DATA HERE		
Dioctyl Sulfosuccinate Sodium Salt CASRN: 577-11-7	Dioctyl disodium sulfosuccinate	Hydrophobic: two 2-ethyl hexyl groups Hydrophilic: Sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C Reference: (2)	6.8×10^{-4} M or 0.03 wt% at 2 Reference (12)
Cationic Surfactants				

Commented [HT13]: Mike's Team still working on

Commented [HT14]: We have a repeat dose study for this; would be Good to get the "Criteria" data ...

Commented [HT15]: This one, we only have an acute study in text, so not really needing the "criteria" data

Commented [KW16]: Updated reference. Duplicate reference in section below was deleted.

Benzalkonium chloride CASRN: 8001-54-5	Quaternary ammonium compounds, alkylbenzyldimethyl, chlorides ChemIDplus: Benzalkonium chloride TSCA:	Hydrophobic: alkyl chains are C12, C14, C16 and C18 and benzyl group Hydrophilic: quaternary nitrogen	BAC tested at 25°C shows surface tension of 37 mN/m concentrations greater than about 4x10-4M Reference (13)	C12: reported values range from 2.3 – 8.5×10^{-3} M @ 25°C or 0.078 – 0.29 wt % C14: 3.7×10^{-4} M or 0.014 wt % C16: 4.2×10^{-5} M @ 23°C or 0.0016 wt % C18: reported values range from 7.1 – 8.5×10^{-6} M @ 23°C or 0.0003 – 0.00036 wt% Reference (12)
Didecyldimethyl ammonium chloride DDAC CASRN 7173-51-5	N-Decyl, N,N-dimethyl-1-decanaminium chloride TSCA: 1-Decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	Hydrophobic: Hydrophilic:	25.82 mN/m at 20 °C, 1 g/L solution *ECHA. Didecyldimethylammonium chloride (7173-51-5). Registered Data Dossier. European Chemicals Agency. Available from, as of Nov 17, 2014* [HYPERLINK "http://echa.europa.eu/search-chemicals"]	CMC is 0.39 g/L at 25 °C /critical micelle concentration/ *ECHA: Didecyldimethylammonium chloride (7173-51-5). Registered Data Dossier. European Chemicals Agency. Available from, as of Nov 17, 2014:* [HYPERLINK "http://echa.europa.eu/search-chemicals"] Possible source of Data

Commented [HT17]: Text says 27

Commented [HT18]: Mike's Team still working on

Commented [HT19]: Physicochemical Properties and Phase Behavior of Didecyldimethylammonium Chloride/Alkyl Polyglycoside Surfactant Mixtures
[HYPERLINK "https://link.springer.com/article/10.1007/s11743-015-1679-5" \l "auth-1"], [HYPERLINK "https://link.springer.com/article/10.1007/s11743-015-1679-5" \l "auth-2"], [HYPERLINK "https://link.springer.com/article/10.1007/s11743-015-1679-5" \l "auth-3"], [HYPERLINK "https://link.springer.com/article/10.1007/s11743-015-1679-5" \l "auth-4"] & [HYPERLINK "https://link.springer.com/article/10.1007/s11743-015-1679-5" \l "auth-5"]
[HYPERLINK "https://link.springer.com/journal/11743"] **volume 18**, pages641–649(2015)

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1. **Schott, H. 1998.** Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339). Journal of Colloid and Interface Science, 205: 496-502. [HYPERLINK "https://doi.org/10.1006/jcis.1998.5721" \t "_blank" \o "Persistent link using digital object identifier"]

2. **Williams, E.F., Woodberry, N.T., Dixon, J.K. 1957.** Purification and surface tension properties of alkyl sodium sulfosuccinates. Journal of Colloid Science, 12: 452-459. [HYPERLINK "https://doi.org/10.1016/0095-8522(57)90048-X" \t "_blank" \o "Persistent link using digital object identifier"]

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Table Y. Surfactant Sub-category Inhalation Toxicity Points of Departure and Human Equivalent Dose Calculation

Surfactant Type	Category Analogue(s)	Exposure Duration/Type	Study POD	Value	Reference	RDDR Model Input Parameters				
						MMAD (µm)	GSD (µm)	RDDR	HEC	
Nonionic	Triton X-100	14-day	LOAEC	5.3 mg/m^3		1.80	1.80	$RDDR_{Pu}$ = 0.564	2.989	
Anionic	oleoyl sarcosine (CASRN 110-25-8),	28-day nose-only inhalation study (OECD Guideline 412)	NOEL (local irritation)	<0.006 mg/L (6 mg/m^3)	[HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]	1.16	2.12	$RDDR_{TotMale}$ = 1.504 $RDDR_{TotFemale}$ = 0.970	>9.024 male >5.8 female	
	dioctyl sodium sulfosuccinate (CASRN 577-11-7)	13-week	LOAEC (blood effects)	4.2 mg/m^3	Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Submission of					

Commented [ST1]: Place holder, Tala is updating this table

Commented [HT2]: WI did male and female separately, but I don't know why?? Add female?

Commented [HT3]: Why not RDDR modelled?

					unpublished data.					
Cationic	DDAC	14-day	NOAEC	0.15 mg/m^3	?					
	DDAC	20 to 21-day	LOAEC* (lung effects)	0.08 mg/m^3	?	1.60	1.85	$RDDR_{Pu/Male}$ = 0.539 $RDDR_{PuFemale}$ = 0.583	0.043 male 0.047 female	
	DDAC	90-day	NOAEC	0.11 mg/m^3	?	0.86	1.63	$RDDR_{PuMale}$ = 0.421 $RDDR_{PuFemale}$ = 0.420	0.046 0.046 female	
	Benzalkonium chloride (BAC)	14-day	LOAEC (nasal effects)	<0.8 mg/m^3	Choi et al., 2020	1.31	1.79	$RDDR_{TotMale}$ = 1.414 $RDDR_{TotFemale}$ = 0.991	<1.13 male <0.79 female	
Amphoteric										
*conservative estimate: effects were not statistically significant										
References: **1.**										

Commented [ST4]: Keith, Tala, and Todd to review each to make decision on selecting one or carrying all 3 through

Tier I

- **Physical-chemical properties to characterize potential for lung exposure and potential for lung effects**
- If respirable aerosols can be generated during manufacturing, processing, or uses and surface tension changes are observed, proceed to Tier II.

Tier II

- ***In vitro* test methods for cytotoxicity, irritation, and/or respiratory toxicity to evaluate toxicity. Combine results with calculated human equivalent doses and predicted human exposures to estimate the Margin of Exposure (MOE).**
- Based on a weight of evidence evaluation, a MOE of greater than 100 would typically indicate further testing is not warranted.

Tier III

- ***In vitro* human airway models with the VitroCell system aerosol exposures to evaluate toxicity. Combine results with calculated human equivalent doses and predicted human exposures to estimate the Margin of Exposure (MOE).**
- Based on a weight of evidence evaluation, a MOE of greater than 100 would typically indicate further testing is not warranted.

Tier IV

- ***In vivo* studies to evaluate effects on the respiratory system in appropriately designed lab animal studies.** Begin with an acute study (OECD TG403); followed, if necessary by a 5-Day inhalation study with a 14-day recovery period to address progression of effects; followed if necessary by a 28-day OECD TG412 inhalation study with a 14-day recovery period. Combine results with calculated human equivalent doses and predicted human exposures to estimate the Margin of Exposure (MOE).

Message

From: Osman-Sypher, Sahar [Sahar_Osman-Sypher@americanchemistry.com]
Sent: 7/22/2020 10:49:31 PM
To: Stedeford, Todd [Stedeford.Todd@epa.gov]
CC: Henry, Tala [Henry.Tala@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]; Irwin, William [Irwin.William@epa.gov]
Subject: General Surfactants Manuscript Draft - July 22 Version 3
Attachments: draft manscript general surfactants - 22 July 2020.ver.3.docx; Tiered Testing Figure rev 22 July 2020.pptm; Table X Example Surfactants in Subcategories_07-19-20.docx; Table Y Haz ID and D-R Table_07-19-20.docx

Importance: High

Hi Todd:

Here is the latest version of the manuscript – July 22, version 3 along with the updated tiered testing figure. The tables are unchanged since the last version I sent you but I'm providing here just for reference.

This version of the manuscript incorporates comments I received today from Rick Becker and Athena Keene. Paul McMullen and Scott Slattery of Scitovation have been added to the author list and have provided me their input which is incorporated. Any minor grammatical or typos have been accepted and others that are substantive or need further discussion left in redline or comment bubbles.

We will use this version for our call tomorrow. Talk to you then.

My power just went out and I'm working off my Wifi box. Hopefully it will return soon!

Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: General Stuff.
Table 3 and 4 could go into Supplemental

Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

Analog or Analogue

Commented [OS2R1]: EPA New Chemicals Working Approach uses "analogue" – did a search and replace

Commented [ST3R1]: Agreed about Tables 3 + 4 into the Supplement

Tala R. Henry[a,‡], Keith Salazar[b,‡], Michael P. Hayes[c], Wayne Kennedy[d], Athena M. Keene[d], Annie Jarabek[e], Stefan Moors[f], Lela Jovanovich[g], Raphael Tremblay[c], Ann Tveit[f], Richard A. Becker[h], Sahar Osman-Sypher[h], Patrick D. McMullen[i], Scott D. Slattery[i], William Irwin[b], Marc Odin[j], and Todd Stedeford[a,]*

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KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc.*), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate (SDS; CASRN 151-21-3; a.k.a., sodium lauryl sulfate), a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol (Nonidet P-40; CASRN 9036-19-5), a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Commented [HT4]: Table?

Commented [OS5R4]: Mike/Wayne to check.

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to “readily penetrate the sandwiched aqueous and lipid barriers of the cornea” (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS6]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled "".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled "".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	CASRN
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of

this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB7]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT8]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA developed and has for decades relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT9]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

l Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.,* a dose or concentration that marks the beginning of a low-dose

Commented [HT10]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Commented [KA11]: We use CSAFs in the Tiered Testing model.

Exposure Assessment

Commented [HT12]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments…include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Commented [ST13]: This is different than particle PDR units; see p. 283 of ChemSTEER manual

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value isvalueis 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Commented [TH14]: Need to write this better and reference.

Commented [TH15]: Need thee quivalent language for liquid droplets/aerosols;

Commented [TH16]: Updated to mist/aerosol as per ChemSTEER

Commented [ST17R16]: Updated

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	$Cm \times b \times h$, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	$Cm = 15$ mg/m^3 $b = 1.25$ m^3/hr $h = 8$ hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: $Cm = KCk \times Ys/Ypel$

Commented [OS18]: There is no Table 5

Commented [TH19]: Have to CHANGE THE PARAMETERS to LIQUID/DROPLETS/AEROSOL

Commented [ST20R19]: Updated

Commented [TH21]: Is this correct for surfactant/mist/aerosol

Commented [ST22R21]: Updated from pp. 283-284 of ChemSTEER manual

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

Commented [HT23]: Say what we do in NC, precisely

Commented [HT24]: (U.S. EPA, 1994).

Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must undertake a risk evaluation process to determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. ~~The MOE equation is presented in Table Y.~~ When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

Commented [RAB25]: Later in the MS HEC is used. So may need to use it here too.

Commented [RAB26]: MOE is not discussed in Table Y

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

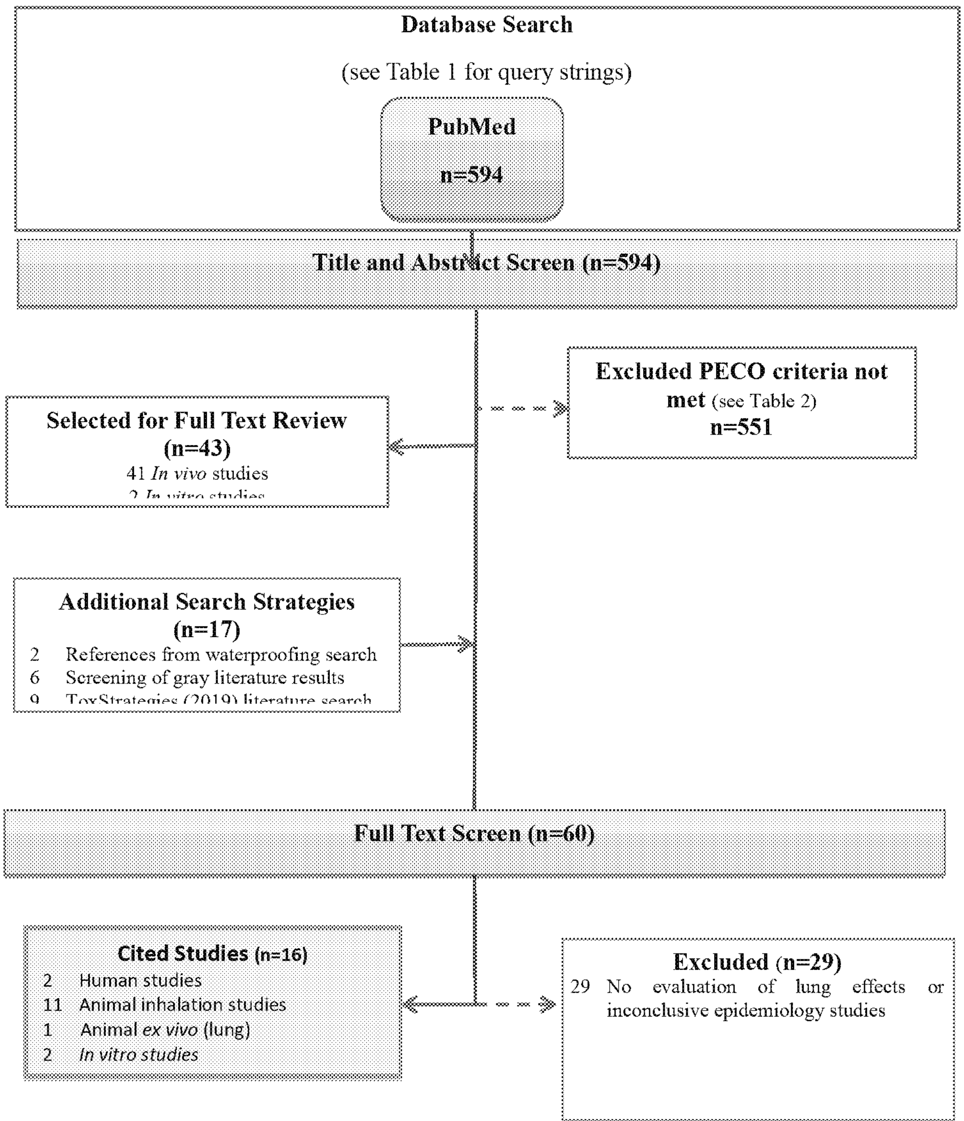
The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

Commented [ST27]: This section needs updating following final disposition of gray lit and Updated Literature Search.

Scheme 1. Literature search and screening flow diagram for surfactants

Commented [ST28]: The tally of Cited and Excluded references from the bottom of the figure includes the PubMed results only. These boxes need to be updated following disposition of 6 studies from the gray lit. search and 9 studies from the Updated Literature Search.



Category Boundaries

Commented [HT29]: REVISIT the general categories paragraphs below the CRITIERA once the TABLE is FINAL

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and ~~either conduct the assessment on the predominant component or on both parts~~the assessment should be conducted on the predominant or both components ~~.~~. A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.

Commented [KA30]: Should this sentence be deleted?

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

Commented [ST31]: Temp?

Commented [OS32R31]: Mike/Wayne to include clarifying text

Commented [HT33]: Need temp here to compare

Commented [HT34]: REMOVE CITE TO EGHE, ADD FOR TWEEN 80

Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

[INSERT TABLE X]

Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants

Commented [ST35]: Not in Mike's Table

Commented [ST36]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"] this paper shows ST to be a lot higher

Commented [ST37]: Concentration?

Commented [ST38]: Temp?

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Commented [ST40]: Add the following, based on Updated Literature Search?
Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS41]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Nonionic Surfactants

Acute Studies

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Commented [OS42]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

Surfactant effect on cell membranes has been studied *in vitro*. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenber et al., (2019) evaluated cytotoxic activity of the of three nonionic polymeric surfactants Polysorbate 20 (Tween 20), Polysorbate 80 (Tween 80) and Poloxamer 188 in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure compared to the classical liquid/liquid (L/L) model. Although less toxicity was observed, significant toxicity of the two Polysorbates (20 and 80) remains when using this more realistic ALI exposure by measuring Lactate Dehydrogenase (LDH) activity. LDH is an intercellular intracellular enzyme present in large amounts in the cytoplasm. The loss of membrane integrity will cause the release of LDH into the extracellular medium. The above results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

Commented [KA43]: I am not sure what is meant here. Needs rewording by author.

Anionic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for several anionic surfactants. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats is using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. The 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Commented [OS44]: Mike/Wayne have indicated that this does not meet the boundary criteria. It is quite insoluble, etc. More information to follow.

Commented [KA45]: Did all animals exposed to 0.05 mg/L survive?

Repeated-Dose Studies

The anionic surfactants oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7) both were identified to have repeated-dose inhalation studies appropriate for deriving a POD.

Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[3]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 µm and the geometric standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses. All test concentrations caused effects at several sites of the respiratory system with indications for local irritation, squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Commented [ST46]: Todd will add these footnotes (i.e., 3 + 4) to EndNote file

[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[4]. There were no statistically significant differences in dosed and control groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Commented [OS47]: Todd to move to Endnote file.

Pulmonary effects have been studied in dogs and/or sheep exposed to anionic surfactant, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). The authors suggested that the observed decrease in pulmonary compliance was due to an increase in surface tension in the alveoli in the presence of detergent. Decreased pulmonary compliance has also been used to indicate loss of natural alveolar surfactant function in animal studies (*e.g.*, Nieman and Bredenberg, 1985). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl

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[4] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity, 6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four 4-day mini-cumulative irritation. Submission of unpublished data by CTFA, 200 pp.

sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). They performed light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol and found no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. The alveolar-capillary barrier consists of the surfactant layer, the alveolar epithelium, the basement membrane and the capillary endothelium. Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, or 4.54 mg/L for 2 hours or 1.36 mg/L for 2 hours and observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Commented [KA48]: Why 1.36 mg/L separated from the other concentrations if the exposure time is 2 h for all concentrations (ie. Why is it not listed as 0.05, 0.09, 0.13, 0.25, 1.36 or 4.54 mg/L for 2 hours)?

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 µm and the geometric standard deviation (GSD) was 2.75 µm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 µm and the GSD was 1.83-1.86 µm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 µm, and the GSD was 1.62-1.65 µm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 µm and the GSD was 1.51 to 2.00 µm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC is more exposed to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole are considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 μg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, cell membrane integrity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels, as well as total amount of protein in cells were analyzed. The results show significant difference in all measurements ~~between~~ for static and dynamic cell growth conditions, following BAC exposure. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Commented [TH49]: This sentence, as written is saying differences between the two growth conditions, not necessarily due to BAC

Commented [OS50R49]: Tala please clarify

Commented [ST51]: William comment: "The text suggests that both static and dynamic systems had effects, but the dynamic system had a more robust response (perhaps due to the physical stresses to the cells in the latter system)."

Dose-Response Analysis: Quantitative Points of Departure (PODs)

Commented [HT52]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

Commented [HT53]: Maybe this goes in the TSCA methods section? Or, because its 'new' to this category, put here?

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 ~~due to application of the RDDR to account~~because ADME differences that would otherwise influence ~~for~~ toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

Commented [HT54]: Need Citation at end of this paragraph...assume it is the OPP Guidance??

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosmetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

Commented [HT55]: Wouldn't have to default if cannot do either?

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST56]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEl9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

Commented [HT57]: This is where WI's comment re: predicting potency, if turned into actual explanation with citations, could be useful.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST58]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

Commented [HT59R58]: Tell us how? Describe the RELATIONSHIP, E.G. increase in ST would increase/decrease toxicity and increase/decrease in CMC is correlated with increases/decreases tox...are there papers on these relationships?

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, $\leq 10\ \mu m$). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

Commented [KA60]: Arch Toxicol . 2017 Nov;91(11):3477-3505. doi: 10.1007/s00204-017-2045-3.

In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
			• Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells • MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

Commented [ST61]: William comment: "Corrosion can be due to acidity, alkalinity or the inherent ability to cause cellular necrosis. Alkaline or acidic compounds can be pH adjusted to neutral values."

Commented [ST62]: Todd to add to EndNote file

CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA63]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as cells from small airways. EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system with normal human stromal fibroblasts, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Commented [OS64]: Scott Slattery Comment: This is a separate product from Epithelix called SmallAir.

Commented [OS65]: Scott Slattery Comment: This is a distinct product called EpiAirwayFT. The standard EpiAirway does not contain stromal fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant. ~~Further, the initial interaction with the epithelium is limited to the apical surface, as would be the case *in vivo*.~~

Commented [OS66]: Scott Slattery Comment: I think this is my statement. Upon re-reading it, I recommend deleting it. There is no requirement that submerged cultures need to be treated on the basal side, so this isn't necessarily a unique feature of ALI exposure.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their

EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019).

Commented [RAB67]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB68]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, ~~In case we may see the~~ physiological responses, other than cytotoxicity, that may be evoked by the surfactant may ~~evoke in the lung.~~

Commented [SM69]: **Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices** Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

~~besides cytotoxicity, interaction with membrane~~be monitored. -One further advantage of PCLS is that ~~you can do~~the PCLS assay can be performed on multiple species ~~in e.g. humans, rats and mice tissues~~ to determine susceptibility~~compare the species~~.

Commented [OS70]: Sentence structure?

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP, *a priori* to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB71]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230015000392"]
[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, ~~these data can be used for informing~~ depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and ~~to~~ for ~~estimate~~ estimating a potency ratio.

Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

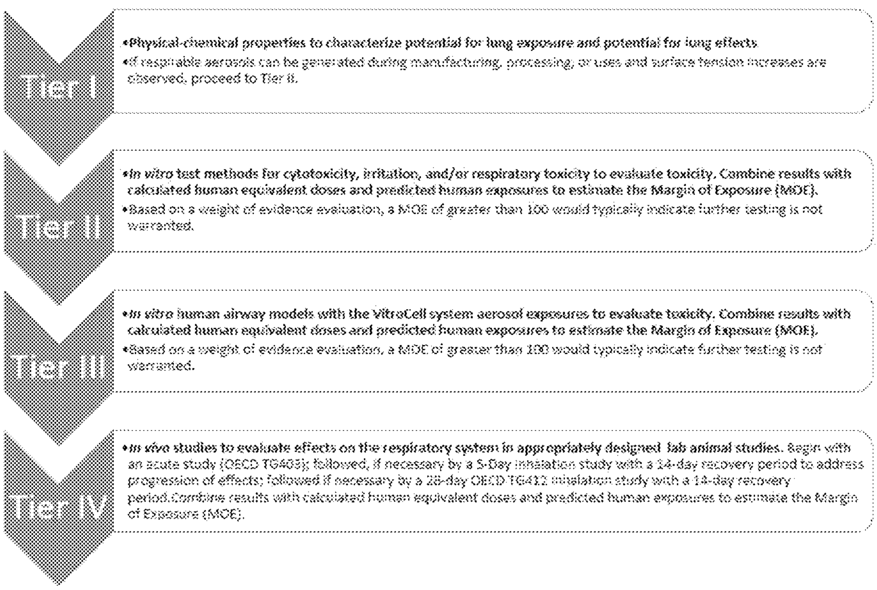
Commented [OS72]: Tala to include some additional text – read across, etc.

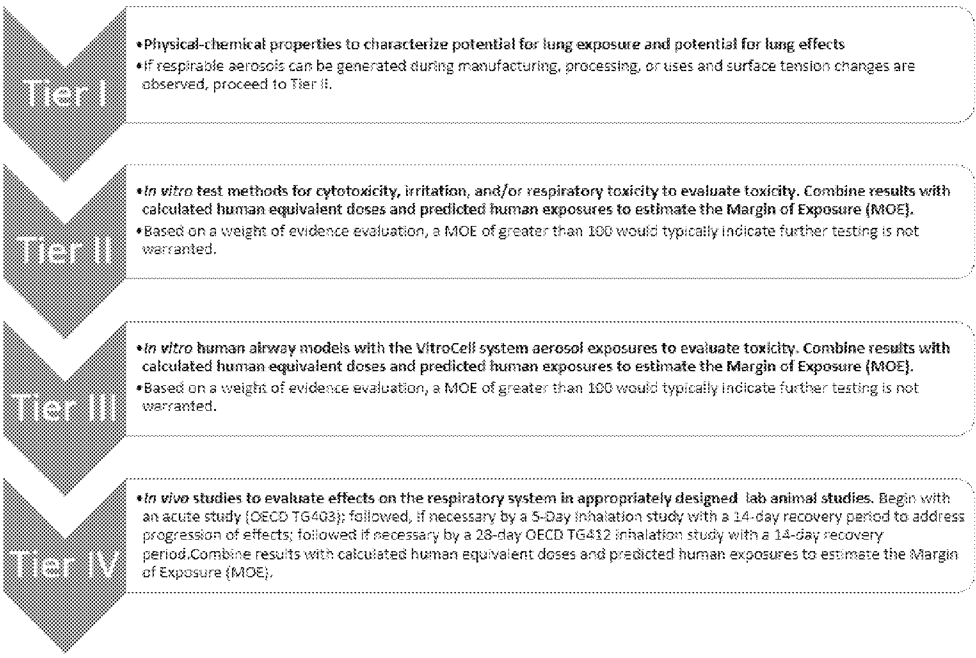
Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.

Commented [ST73]: William comment: "Recommend replacing the word increases with changes in Tier I. Surfactants lower the surface tension of water, but can raise the surface tension of lung fluid by interfering with the natural lung surfactants."





Tier I—~~Use p~~Physical-chemical properties~~ to characterize lung exposure/disruption~~

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If ~~the new chemical substance meets the Surfactant Criteria and~~ respirable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods ~~can~~ may provide potentially useful information with towards informing MIEs and CLEs~~:~~. In order to determine the best approach for *in vitro/ex vivo* testing, a pre-notif~~ication~~ice consultation with EPA should be considered. ~~Currently~~, given that none of the following studies are validated to determine ~~surfactant~~ lung toxicity, induced by surfactants. In general, the testing approach should include a combination of assays should be performed, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro/ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro/ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc.*

Pulmonary surfactant interaction/loss of function

Commented [OS74]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST75R74]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

~~Cytotoxicity~~*General cytotoxicity*

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of these assays may be used to determine a starting point to calculate a ~~human-equivalent concentration (~~modified POD_{HEC}~~)~~ using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified ~~based on the in vitro or ex vivo~~from the assays ~~used will be used~~should be used to calculate a ~~point of departure~~POD using ~~Benchmark Dose~~BMD modeling, when possible, ~~analysis using~~with the $BMDCL_{(1SD)}$ metric. This metric is based on the ~~For the 2018 US EPA FIFRA inhalation Scientific Advisory Panel meeting, a~~ benchmark response (BMR) of one standard deviation ~~was~~ suggested for *in vitro* assays (a ~14.9% change from the

Commented [ST76]: William comment: "If the metric is MTT, cell death or cytotoxicity, then an EC50 value is not very sensitive and a lower BMR should be considered (i.e. BMDL10)."

Commented [OS77R76]: William to provide citation control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ~~-~~([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, ~~in highly cited work~~ the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). The *in vitro* ~~point of departure~~POD can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols. -In those situations where data are not amenable to BMD modeling, due to ~~Considering that certain models may not be~~assays that are not designed to provide a ~~dose~~concentration response data and/or lack sufficient granularity, ~~alternatively~~ the *in- vitro* testing ~~dose~~concentration level ~~could~~should be determined based on the expected HEC (taking into account the necessary MOE) to ensure ~~so~~ that the *in vitro* data ~~is~~are generated in a concentration range relevant ~~of~~to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in- vitro* testing exposure dose.

Commented [OS78]: Raphael addition

~~After~~When the data are amenable to calculating ~~the~~an HEC ~~is calculated~~, ~~various~~the relevant routes of exposure ~~must~~should be considered, based on the conditions of use. A margin of exposure may then be determined by dividing the HEC by the estimated exposure. ~~. For consumer exposures, ExpoCast or a similar model can be used. For industrial and occupational scenarios, exposure estimates will need to be tailored to the application and context. Additionally, when deciding if *in vivo* testing is needed, PPE and other methods to limit exposure should be considered.~~

Commented [RAB79]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

Formatted: Highlight

~~The margin of exposure can be determined by dividing the HEC by the estimated exposure. Appropriate risk assessment uncertainty factors are used to safely account for inter- and intra-species factors, and additional uncertainty factors should be added to account for in vitro exposure. A margin of exposure of greater than 100 may mean that in vivo testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (in vitro or in vivo) is needed. Tier III and IV testing should only be done in consultation with EPA. Even if additional in vivo testing is needed, these assays can be used to determine a starting dose, potentially reducing animal testing.~~

Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the 3 assays, will drive the determination of "greater" or "comparable" toxicity, whereas negative results in all 3 assays will drive the determination of "lower" toxicity, as described below.

Commented [RAB80]: Its not clear how MOE fits into these decision criteria. I inserted draft text below – highlighted -- as a suggestion – please review and revise as needed

If the new chemical substance exhibits greater toxicity to the positive control in one of the evaluated assays, per the study method criteria, proceed to Tier III.

If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the margin of exposures for specific conditions of manufacturing, formulation and use to ~~and~~

consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then reassess risks using the modified POD_{HEC}, using MOE as the risk metric. If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

~~In vitro respiratory toxicity~~

- ~~Precision-cut lung slice test etc. as described by Hess et al., (2016)~~
- ~~In vitro alveolar macrophage assay as described by Weinman et al., (2016)~~
- ~~In vitro lung surfactant inhibition as described by Sorli et al., (2017)~~
- ~~Other in vitro lung toxicity assays as described above~~

~~In order to determine the best approach for in vitro testing, a prenotification consultation with EPA should be considered. Currently, none of the studies are validated to determine surfactant lung toxicity. However, data from these studies may provide detail information from e.g. test systems mentioned to evaluate substance behavior and may also give a first assumption on potency. Each of these assays may be used to determine a starting point to calculate a human equivalent concentration (HEC) using in vitro to in vivo extrapolation (IVIVE). The most~~

Commented [OS81]: Raphael: Delete if taken off earlier section.

~~sensitive of the endpoint based on the in vitro or ex vivo assays used will be used to calculate a point of departure using Benchmark Dose analysis using the BMDL₁₀ metric. For the 2018 US EPA FIFRA inhalation Scientific Advisory Panel meeting, a benchmark response (BMR) of one standard deviation was suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay.~~ [HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]~~). However, in highly cited work the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). The *in vitro* point of departure can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols. Considering that certain models may not be designed to provide a dose response and/or lack granularity, alternatively the in-vitro testing dose level could be determined based on the expected HEC (taking into account the necessary MOE) so that in-vitro data is generated in concentration range relevant of the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard in-vitro testing exposure dose.~~

Commented [ST82]: William comment: "If the metric is MTT, cell death or cytotoxicity, then an EC50 value is not very sensitive and a lower BMR should be considered (i.e. BMDL10)."

Commented [OS83R82]: William to provide citation

Commented [OS84]: Raphael addition

~~After the HEC is calculated, various routes of exposure must be considered. For consumer exposures, ExpoCast or a similar model can be used. For industrial and occupational scenarios, exposure estimates will need to be tailored to the application and context. Additionally, when deciding if *in vivo* testing is needed, PPE and other methods to limit exposure should be considered.~~

~~The margin of exposure can be determined by dividing the HEC by the estimated exposure. Appropriate risk assessment uncertainty factors are used to safely account for inter- and intra-~~

~~species factors, and additional uncertainty factors should be added to account for in vitro exposure. A margin of exposure of greater than 100 may mean that in vivo testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (in vitro or in vivo) is needed. Tier III and IV testing should only be done in consultation with EPA. Even if additional in vivo testing is needed, these assays can be used to determine a starting dose, potentially reducing animal testing.~~

~~Note: If substance is found to be corrosive within in vitro or in vivo tests, avoid further in vivo testing. If the substance is a severe irritant based on in vitro testing, appropriate Testing in Tier III should be chosen.~~

Tier III – Human Airway Models/PCLS Assay

- Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures

In vitro to *in vivo* extrapolation to develop a HEC in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the HEC by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the HEC can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these substances leading to impacts on lung surfactant or cell membranes. Because these effects are

Commented [KA85]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

Commented [OS86]: Stay consistent AOP not MoA – search throughout related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf). A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (*e.g.*, engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.

Tier IV—*In vivo* studies

Note that a prenotification consultation with EPA should be considered prior to undertaking any Tier IV testing.

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. ~~Proceed to step 2 if LOAEC <2000 mg/m³~~. As described above, the HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is > 100, further testing is not needed.

Commented [KA87]: Can also reference Klein et al. Hazard ID using short term inhalation studies 2012

- Step 2: 5-Day inhalation study with a 14-day recovery period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is > 100, further testing is not needed.

- Step 3: OECD TG 412**: 28-day inhalation study in rats with a 14-day recovery period.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA88]: Alarie, Y., G.D. Nielsen, and M.M. Sch bioassays for evaluation of indoor air quality *Quality Handbook*. Spengler, J.D., J.M J.F. McCarthy (eds.). New York: McGra pp 23.21-23.49.

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

ACKNOWLEDGMENT

Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Table X. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cation
Subcategorization

Chemical Name in Text	Other Relevant Names	Criteria 1	Criteria 2	Criteria 3
Nonionic Surfactants				
Octoxynol 9 CASRN 9002-93-1	Triton X-100 Octylphenol ethoxylate 4-1,1,3,3-tetramethylbutylphenol ethoxylated ChemID*plus*: Octoxynol 9 IUPAC: 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol TSCA: Poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	Hydrophobic: octylphenol group Hydrophilic: polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C Reference: (1)	CMC is 0.17 or 0.017 wt% Reference: (1
~~Ethylene glycol n-hexyl ether (EGHE)~~ ~~CASRN 112-25-4~~	~~Ethylene glycol monohexyl ether~~ ~~ChemIDplus: 2-hexyoxyethanol~~ ~~IUPAC: 2-(hexyloxy)ethan-1-ol~~ ~~TSCA: Ethanol, 2-(hexyloxy)-~~	~~Hydrophobic: hexoyl group~~ ~~Hydrophilic: ethyoxylate group~~	~~~33.5 mN/m at 10 g/L (1.0 wt%) and 25°C~~ ~~Reference: DOW?? from TEXT~~	
Tyloxapol Defomaire Alevaire CASRN 25301-02-4	Triton WR 1339 Formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol	Hydrophobic: multiple octyl phenol groups Hydrophilic: multiple	~37 mN/m at 5 g/L (0.5 wt%) and 25°C Reference: (1)	CMC is 0.038 g/L or 0.0038 wt% Reference: (1

Commented [HT1]: DONE:
1)Removed EGHE
2)Moved DDAO to Nonionic and put note about pH
3)Got full references

TO DO
1) Decide on 'other relvant names/ how many/which ones [im thinking ChemIDplus and TSCA...couldn't find IUPAC for all]
2) Cross walk Examples with TEXT and Studies having PODsClean up how Criteria 2 & 3 are presented; they vary throughout
3) DDAC criteria
4) SDS criteria
5) Format for citing the Refs in Table

Commented [HT3]: Verified names/synonyms and CASRN in ChemIDplus.
Provide ChemIDplus name as "reference"?
Provide ChemIDplus link? (could get old)
Should we streamline this table and put the details on names, etc in a supplemental?

References: can a table have its own references? OR do they have to be put into the overall Reference Section? (journal rules)

The Reference citations are incomplete; will need full cites. What is the Journal citation style?

Commented [HT2]: Went with using the "Chemical Name in Text" as the anchoring name, for transparency/crosswalk with the tox studies.
"other relevant names" are from ChemIDPlus (trade or common mostly from what was orginally provided...there can be A LOT of synonyms).
Put in IUPAC and TSCA names just to have a 'common denominator', but could not find for all...e.g., a couple of these chems appear NOT to be on TSCA Inventory

Commented [HT4]: Check spelling names: say hexyloxy or hexyloxy; none have 'oyl'

Commented [HT5]: check

Commented [HT6]: No CMC

Commented [KW7]: Not a surfactant. Need to remove

Commented [HT8]: NOT in ChemIDplus as synonym

Commented [HT9]: NOT @ 20 deg...is there a 'conversion' method?

	ChemID*plus*: Tyloxapol TSCA: Not on TSCA Inventory	polyoxyethylene (9) units		
Polyoxyethylene-10-oleyl ether CASRN 9004-98-2	$C_{18:1}E_{10}$ Oleyl ethoxylate Oleth-10 Brij 97 ChemID*plus*: Polyoxyl 10 oleyl ether TSCA: Poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	Hydrophobic: oleyl group Hydrophilic: polyoxyethylene (10) unit	35.17 mN/m at CMC and 25°C from Table 1 ~37 mN/m at CMC and 25°C from Fig 1 Reference: (8)	$4x10^{-5}$ M at 25°C or 0.028 wt % Reference: (8)
Polyoxyethylene-10-dodecyl ether CASRN: 9002-92-0	$C_{12}E_{10}$ Polyethylene glycol monododecyl ether Polyoxyethylene (10) lauryl ether ChemID*plus*: Dodecyl alcohol, ethoxylated TSCA: Poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	Hydrophobic: dodecyl group Hydrophilic: polyoxyethylene (10) unit	C12E9: 36 mN/m at 23°C C12E12: 32 mN/m at 23°C Reference: (10)	$12.7x10^{-6}$ M at 30°C or 0.0008 wt % Reference (9) Also, C12E9 at $1x10^{-6}$ M at 23°C and C12E12 at $1.4X10^{-6}$ M at 23°C Reference: (10)
Polysorbate 20 Tween 20 CASRN 9005-64-5	Polyoxyethylene (20) sorbitan monolaurate ChemID*plus*: Polysorbate 20	Hydrophobic: dodecanoyl group Hydrophilic: sorbitan	38 mN/m at the CMC and 21°C Reference: (3)	$8.04x10^{-5}$ M at 21°C or 0.001 wt % Reference: (3)

Commented [HT10]: CehmID Plus shows Oleth 9; but not 10 as synonym

	TSCA: Sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	polyoxyethylene (20) unit		
Polysorbate 80 Tween 80 CASRN 9005-65-6	Polyoxyethylene (20) sorbitan monooleate ChemID*plus*: Polysorbate 80 TSCA: Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	Hydrophobic: octadecenoyl group Hydrophilic: sorbitan polyoxyethylene (20) unit	37.96 mN/m at 0.5 wt % Reference: (5)	1.5×10^{-5} M at 25°C or 0.00 % Reference: (4)
Poloxamer 188 CASRN 691397-13-4	Pluronic F-68 ChemID*plus*: Poloxalene IUPAC: 2-[2-(2-hydroxyethoxy)propoxyl]ethanol Oxirane, 2-methyl-, polymer with oxirane, triblock	Hydrophobic: polyoxypropylene (27) unit Hydrophilic: two polyoxyethylene (80) units	42-44 mN/m Reference: (6)	4.8×10^{-4} M at 37°C or 0.4 wt % Reference: (7)
N,N-Dimethyl-dodecylamine-N-oxide ($C_{12}AO$) CASRN 1643-20-5 Zwitterionic: At pH 7, 90% expected to be nonionic; only small amount cationic	1-Dodecanamine, *N,N*-dimethyl-, *N*-oxide Lauryl dimethylamine **oxide** ChemID*plus*: Lauramine oxide TSCA: 1-Dodecanamine, N,N-dimethyl-, N-oxide	Hydrophobic: dodecyl group Hydrophilic: amine oxide unit	32.6 mN/m at CMC Reference: (11)	1.7×10^{-3} M or 0.039 wt % Reference: (11) Mukerjee et al. also report values from 1×10^{-5} M to 5.5×10^{-5} M at 25°C Reference: (2 and 12)

Commented [HT11]: Temp//doesn't have g/L like others

Commented [HT12]: ChemIDplus indicates: N,N-Dimethyl-1-dodecanamine-N-oxide
And
N,N-Dimethyldodecylamine oxide (no 3rd N)